

Future Research Directions to Characterize Environmental Mutagens in Highly Polluted Areas

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Population monitoring using methods of molecular epidemiology combined with reliable data on exposure is an extremely powerful approach to determine the effect of mutagens on human populations. Although human blood and urine have traditionally been used for biomonitoring, an increase in the use of placental and buccal smear samples should be expected. As biomarkers of exposure, DNA strand breaks and hemoglobin and albumin adducts seem to be most sensitive. As biomarkers of response, cytogenetic analysis determining chromosome aberrations or micronuclei has been widely used. Additional information can be obtained by using the chromosome painting technique and by determining gene mutations at the *hprt* locus; however, epidemiological studies exhibiting a relationship between these biomarkers and environmental pollution are still lacking. The use of sperm to analyze the effect of environmental mutagens in germ cells (e.g., sperm morphology and sperm aneuploidy) should be encouraged. The determination of susceptibility by analyzing genetic polymorphism, which is responsible for individual differences in the biotransformation of mutagens and carcinogens, will gain importance for risk assessment. Future research should include validating molecular methods, studying adaptive response to chemical carcinogens, and studying the modulatory effect of antioxidants, as well as the effect of carcinogens on immunity. — *Environ Health Perspect* 104(Suppl 3):603–607 (1996)

Key words: biomarkers of exposure, biomarkers of response, biomarkers of susceptibility, adaptive response, antioxidants, genotoxicity and immunity

Introduction

It has been generally known that pollution in eastern Europe greatly exceeded the standards in western Europe and the United States. Subjects in these regions believed that pollution from power plants was responsible for higher mortality, cancer incidence, birth defects, and immune deficiency in their children. There was a similar situation in industrial zones of Poland (1,2), East Germany, and Czechoslovakia (3). In the past, there was not enough experience with biomarkers to evaluate environmental exposure; nevertheless, any

results relating to environmental pollution and human health were not usually allowed to be published. Furthermore, methodology for this type of study was not on the contemporary level. With political changes in the fall of 1989, we suggested that these regions could be utilized to study the application of human biomarkers and their usefulness for risk assessment (4). Using internationally acceptable analytical techniques, substantial differences between original opinion and new scientific data were noticed. Experience from these

studies allows us to comment on the use of biomarkers for human biomonitoring.

It is necessary to consider exposure evaluation for the use of any biomarker. The impact of pollutants should be evaluated from all expected sources, usually air, water, and food. If we evaluate the effect of air pollution, not only ambient but also personal exposure should be determined. Many papers on this topic lack concurrent data on air pollution measured in the same period when blood samples were collected (1,2). Pollution in eastern Europe changes significantly with changes in economy, which alters demand for electricity; therefore, it is not acceptable to use the results of measurement in one year for the interpretation of biomarkers used several years later.

To characterize a population, we need sufficient information about lifestyle. We should know if the diet contains chemicals that may act as enzyme inducers or mutagens. Modulatory effects of antioxidants have been observed repeatedly (5–8); therefore, plasma levels of vitamins C, A, and E should be determined in order to provide information on the quality of diet and on the supply of antioxidants. The effect of tobacco smoking on various biomarkers has already been proven (9–11). The rate of smoking is higher in polluted regions and in lower socioeconomic groups. Determining cotinine levels in urine or other fluids has become the method of choice to check the accuracy of answers in questionnaires for active and passive smoking (12). Alcohol consumption in higher doses may act as a modulator or, through its metabolism, increase the level of free radicals (13,14). Hartmann et al. (15) recently published data on a similar effect from exhaustive physical exercise.

Biomarkers

Biomarkers in molecular epidemiology are usually classified into three groups: biomarkers of exposure, biomarkers of response or effects, and biomarkers of susceptibility.

Biomarkers of Exposure

Biomarkers of exposure include biomarkers of internal dose and biological effective dose. The detection of mutagenic urine using new bacterial strains simultaneously with analysis for genetic polymorphism (11) may indicate further relevance for these techniques.

To determine the biological effective dose, DNA adducts and protein adducts

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Abbreviations used: PAHs, polycyclic aromatic hydrocarbons; SCE, sister chromatid exchanges; FISH, fluorescent *in situ* hybridization; *GSTM1*, glutathione S-transferase M1 gene.

seem to be the methods of choice (16). In fact, until now, the only method used to evaluate the impact of exposure to polycyclic aromatic hydrocarbons (PAHs) has been the ^{32}P -postlabeling technique, which was used together with ambient air and personal exposure monitoring (12,17). White blood cells (WBC) and lymphocytes are the cells of choice for these studies. A promising method for human biomonitoring is the comet assay, which seems to be very sensitive, but it still waits for international validation (18).

Biomarkers of Response

Biomarkers of response involve the determination of chromosomal and gene mutations. Certainly the most popular approach is to study chromosome aberrations (19). Their importance is increased by the Nordic study, which demonstrates the relationship between significantly increased frequency of chromosome aberrations and the risk of cancer (20,21). On the other hand, chromosome aberrations are unspecific, corresponding to the complex effect of environment, occupation, and lifestyle. Sister chromatid exchanges (SCE) seem to be sensitive, especially to cigarette smoking (9). New potential may be expected from the determination of high frequency cells, but its significance to environmental pollution is still lacking (22). Analysis of micronuclei is advocated to determine also the effect of aneugens. It is certainly a useful method to evaluate the impact of radiation exposure, as well as the effect of aging (23). A new approach, FISH (fluorescent *in situ* hybridization), can determine stable chromosome exchanges (24,25).

The most sensitive gene mutation seems to be the *HPRT* mutation (26). Recently, Albertini et al. (26) and Ammenheuser et al. (27) indicated the effect of transplacental transfer from smoking mothers, as well as the effect of social environment, but studies of the relationship between *HPRT* mutations and measured environmental exposure are still to be done (28). The glycophorin A assay and oncogene activation were not proven to be satisfactorily sensitive in a sufficiently large study.

Special attention should be given to the effects of environmental exposure to gametes. Wright (29) has repeatedly recommended that DNA adducts, single strand breaks in DNA (comet assay), aneuploidy by FISH, and mutations by restriction fragment length polymorphism should be measured in sperm. Due to the difficulties with sampling, such studies are probably

only in the early stages of preparation. For example, Selevan et al. (30) showed the relationship between sperm morphology and air pollution when SO_2 was used as a surrogate measure of all air pollutants (Figure 1). In polluted districts after the winter season, air pollution affected the percentage of morphologically normal sperm and sperm heads.

Another important area involves studies on transplacental transfer of toxicants. It may be speculated that the defense mechanism of the mother may be significantly affected by her lifestyle. For studying efficiency of transplacental transfer, all biomarkers with proven validity should be used.

Recently Autrup et al. (31) studied the presence of PAH-albumin adduct in human tissues. They observed a relationship between the adduct levels in the mother and in umbilical cord blood with a ratio of approximately 1.3. The level of exposure was evaluated according to residence and the use of transport, characterized as city, suburban, and rural area. They pointed out the significance of public transport (length of time spent in transport) to the adduct level. The lowest adduct level was observed in a suburban area. Tavares et al. (32) showed the effect of smoking on hydroxyethylvaline adducts of hemoglobin, with ratios (adduct level in cord blood vs adduct level in venous blood) of approximately 2.4 in smoking mothers compared with 1.5 in nonsmoking mothers. These data indicate a significant capacity of maternal tissues to protect fetuses against the effect of pollutants. Furthermore, we may expect that, with higher concentrations of pollutants, the mother-fetus interactions are more effective. The defense mechanism of transplacental transfer should be explored in the future to learn if environmental exposure can induce measurable changes.

Biomarkers of Susceptibility

A very promising approach for epidemiology research seems to be the detection of biomarkers of susceptibility (33), the study of DNA repair and genetic polymorphism. DNA repair is of key significance if genetic damage is really induced. In the past, unscheduled DNA synthesis was used to evaluate DNA repair capacity. This method is not very sensitive and has large interindividual variability (34). A similar approach corresponds to the challenge assay, which advocates the use of a known dose of mutagen to induce chromosome aberrations in human peripheral lymphocytes (35).

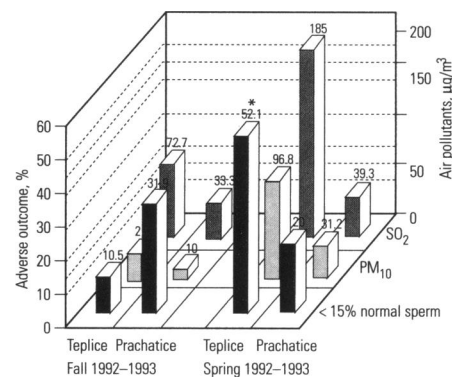


Figure 1. Sperm morphology and level of air pollutants for Teplice, a polluted district, and Prachaticte, a control district. PM₁₀, particulate matter less than 10 μm aerodynamic diameter. * $p < 0.05$. SO₂, mean 90 days. PM₁₀, mean 90 days.

A new method of evaluating DNA repair is the comet assay. Green et al. (8) observed the effect of diet and vitamin C on DNA single-strand breaks. It may be hypothesized that the antioxidant level speeds up biotransformation of mutagens and improves human DNA repair capacity. Collins et al. (36,37) used the comet assay and endonucleases specific for oxidized pyrimidines to detect oxidative damage.

Molecular technology brought a new qualitative step to study genetic polymorphism, which is responsible for the genetically based metabolic susceptibility to carcinogens. More and more data may define the relationship between genotypes and different types of cancer. It is certainly a very complex process and we can explore only a part of the important genotype. We should ask ourselves if we are entitled to use the knowledge on genetic polymorphism for preventive measures in certain groups of individuals. Is it ethically acceptable? Should the genotypes of workers be studied as a prerequisite to working in an environment with a high exposure to carcinogens, e.g., coke ovens, PAHs. We should try to come to a consensus when the use of a new knowledge is generally acceptable, and we need to know which genetic-environmental interactions are worse. What is the impact of higher exposure? What may be the impact of combined effects of several carcinogens?

Using ^{32}P -postlabeling to study the DNA adducts in placenta, Topinka et al. (38) found no significant difference of DNA adduct levels between two districts; this corresponds to a similar exposure to carcinogenic PAHs but different concentrations of SO₂ and NO_x. If the population

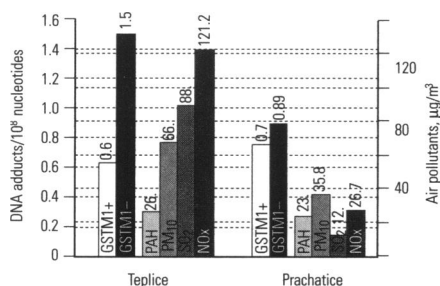


Figure 2. Effect of *GSTM1* on DNA adducts in placenta in Teplice, a polluted district, and Prachatice, a control district. Air pollution concentrations are averages for October to December 1994 when placenta samples were collected.

was divided according to glutathione *S*-transferase M1 (*GSTM1*) genotype, individuals with the *GSTM1* null genotype had a significantly increased DNA adduct level in the polluted district (Figure 2). This result implicates a possibility that sensitivity of subjects may be seen especially with high exposure to mutagens. This is consistent with the data of Hirvonen et al. (11) on mutagenicity of urine in smokers compared to nonsmokers in which the *GSTM1* null genotype affected mutagenicity in smokers only. On the other hand, Oesch et al. (39) suggested that simultaneous smoking decreases additional DNA damage from other exposures. Vineis and Martone (40) speculated that the effect of genotype is more pronounced at low doses and that individual susceptibility is irrelevant under exceptionally high-exposure conditions. Future epidemiological studies should test which of these hypotheses may be correct in the evaluation of the effect of complex mixtures in environmental exposure.

Future Research Directions

Validation of Molecular Methods

Epidemiologists usually acknowledge the limitations of biomarkers in cancer epidemiology (41); therefore, to use molecular methods for human biomonitoring, we need to validate the usefulness of biomarkers. A role of international agencies such as the International Agency for Research on Cancer (IARC), the United Nations

Environment Programme, the World Health Organization, the International Atomic Energy Agency, DG XII EC, the National Institute of Environmental Health Sciences, and the U.S. Environmental Protection Agency is to stimulate processes of validation for the most promising methods. One example is the EU-IARC activity on interlaboratory standardization and validation of DNA adduct postlabeling methods for human studies (42). We should put forward proposals for methods that seem to be most promising and which should be validated, e.g., the comet assay and protein adducts and genotypes of carcinogen metabolism.

Adaptive Response

Many years ago, Wolff et al. (43) demonstrated adaptive response for ionizing radiation. Experience from eastern Europe indicates that the impact of pollution on human health is less dramatic than the sum of pollutants in these regions. Forests have died but humans survived. Similar unexpected results were observed by Natarajan et al. (44) among Indians in Argentina who were exposed to high concentrations of arsenic in drinking water but did not show any signs of chronic arsenic poisoning. It is also known that coke-oven workers are exposed to high concentrations of PAHs, but their injuries do not correspond to the level of carcinogen exposure. These examples postulate adaptive response in human populations, a response that is certainly determined by genotype. But lifestyle probably plays a very important role in adaptive response. We should study how we can increase adaptive response if mechanisms are available.

Modulatory Effects of Antioxidants

Several years ago we observed the effect of ascorbic acid and α -tocopherol on the frequency of chromosome aberrations, lipid peroxidation, and unscheduled DNA synthesis in groups occupationally exposed to PAHs (5), in chronic alcoholics, and in an aging population (34). We believed that the effect of exposure to some carcinogens or free radicals could be decreased with higher doses of antioxidants by a long-term

supplementation. This idea concerning the significance of vitamin C was later confirmed using the comet assay by Green et al. (8) and the significance of α -tocopherol confirmed by Hartmann et al. (45).

In the chemical industry, it is technologically difficult to prevent all exposures of workers to carcinogens. Thus, under these conditions, exposed workers should be supplemented with antioxidants. We should try to evaluate which antioxidants and what doses can be recommended.

Even present knowledge indicates the importance of lifestyle to carcinogenicity. It may be very effective and profitable to implement the use of antioxidants in the general population in highly polluted areas.

Relationship to Immunity

Genotoxicity and immunity were for a long time understood as two separate processes. Verdina et al. (46) suggested that specific immunological responses induced by recurrent carcinogen exposure may exert a modulatory effect and act as a relevant host factor in chemical carcinogenesis. It is expected that carcinogen can produce specific antibodies against carcinogen, which may decrease the level of DNA damage (e.g., DNA adducts) in immunized subjects. This elegant idea should be thoroughly explored.

Conclusion

In using molecular methods for human biomonitoring, we should not expect that only one method is sufficient. To cover the spectrum of biomarkers, we can succeed only with efficient cooperation among various specialists (epidemiologists, analytical chemists, biochemists, molecular biologists, cytogeneticists, hygienists, etc.). This task becomes the real ground for international collaboration. We should try to characterize possible highly polluted areas and formulate hypotheses about why such studies should be performed. Such regions could become models to test the validity of molecular methods and their possible interpretation. Fruits from this activity will be used to improve our scientific knowledge and to improve human health.

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